EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure for Performance Verification of Autoclaves

SOP Number: QC-13-02

Date Revised: 08-28-02

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1.0 SCOPE AND APPLICATION:

1.1 This protocol describes procedures for verifying the performance of the autoclaves. Small changes in temperature within the autoclave produce a large effect on the time required to achieve the predetermined process parameters. It is therefore critical to ensure that the autoclaves are operating within acceptable limits (see ref. 15.1 and 15.2).

2.0 DEFINITIONS:

2.1 NIST = National Institute of Standards and Technology

2.2 Completed Cycle = a complete autoclave cycle includes the recommended 10 minute wait period (indicated on the LED screen on the autoclave) once the door has been cracked open (not greater than one inch). During this time, the steam is allowed to escape and the contents allowed to cool and become acclimated to the ambient temperature to minimize thermal shock, especially to liquids in glass containers.

3.0 HEALTH AND SAFETY:

3.1 Laboratory personnel have been trained on the proper use of the autoclaves. Documentation of training can be found in the training file for each employee.

4.0 CAUTIONS:

4.1 Since autoclaves use high temperatures, it is necessary to exercise extreme caution around the device and its associated plumbing. High-temperature surfaces can be encountered even when the device is not in a sterilizing cycle.

5.0 INTERFERENCES:

5.1 The maximum registering thermometers should be reset prior to each use as described in section 10.

5.2 Shake the thermometer until the column registers 110°C or lower.

5.3 The thermometer should be allowed to cool to ambient temperature

before it is read. Hold thermometer in an upright position for reading, and only after it has cooled to ambient temperature, or you will obtain a falsely high reading.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Raven Biological Laboratories ProSpore Biological Indicator Ampules with 10^6 spores of *Bacillus stearothermophilus* (ATCC #7953)

7.2 SPSmedical Chemical Indicator Strips

7.3 Incubator with temperature at 55°C ± 1°C

7.4 Autoclave #1 located in room B206, Amsco Eagle 3000 Scientific Series, Model E3031-S-1, Serial No. 0105898-25

7.5 Autoclave #2 located in room B204, Amsco Eagle 3000 Scientific Series, Model E3031-S-1, Serial No. 0108298-11

7.6 Autoclave #3 located in room B207, Amsco Century Series Scientific Gravity Sterilizer (16×16×26"), Model SG-116, Serial No. 0121198-15

7.7 Autoclave #4 located in room B202, Amsco Century Series Scientific Gravity Sterilizer (20×20×26"), Model SG-120, Serial No. 0103000-16

7.8 Certified Maximum Registering Thermometers (scale range 80-135°C)

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Maximum registering thermometers must be calibrated against a NIST traceable thermometer and certified annually (see SOP EQ-02, Thermometers).

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Biological indicator ampules (sealed spore ampules containing spores in liquid culture media) must be stored according to manufacturer's specifications to insure shelf life. Care must be taken to insure that they are unpacked immediately upon arrival and placed in the refrigerator.

10.0 PROCEDURE AND ANALYSIS:

10.1 Summary. Autoclave performance will be verified by examining the autoclave printout and through the use of maximum registering thermometers, chemical indicator strips and biological ampules. Specifically, the printout, thermometer, and chemical strip will be used with each daily sterilization run. The printout, thermometers, chemical strips, and biological ampules will be used concurrently for the performance verification. Performance verification will be performed on a monthly and quarterly basis, as described in section 10.3. Refer to Table 10.1 for a summary of the performance verification practices.

Table 10.1

Performance Verification Method	Frequency of Use	
	Daily (per load)	Monthly
Autoclave Printout	X	X
Max Thermometer	X	X
Chemical Strip	X	X
Biological Ampule		X

10.2 Performance Verification of Daily Runs.

10.2.1 Autoclave Printout. For each run, record the minimum and maximum temperatures achieved during the "sterilize" portion of the cycle as indicated on the autoclave printer readout on the appropriate form (see 16.0). The probe that monitors the temperatures indicated on the Autoclave Printout (both Max. and Min.) is located at the chamber drain. The chamber drain is the coolest location within the

autoclave.

10.2.2 Maximum Registering Thermometer. A maximum registering thermometer is used for each daily autoclave run. Place the thermometer upright in a beaker and place the beaker in the autoclave pan along with the items to be processed. The beaker should be placed in a position central to the contents of the run.

10.2.2.1 Record the results from the thermometer on the appropriate form (see 16.0). The thermometer should be allowed to cool to ambient temperature before it is read (see 5.3).

10.2.2.2 Reset the maximum registering thermometer prior to each use by "shaking" the thermometer as you would a fever thermometer. This will force the mercury through the constriction located above the bulb (see 14.1).

10.2.3 Chemical Indicator Strip. Place the strip flat on top of the beaker that contains the maximum registering thermometer. Do not place the strip upright within the beaker that contains the maximum registering thermometer.

10.2.3.1 Record the results from the chemical indicator strips on the appropriate form (see 16.0).

10.3 Monthly Performance Verification and Use of Biological Ampules.

10.3.1 Verify kill load liquid cycles (three hour cycles used to sterilize both solid and liquid media and contaminated trash generated during testing) in autoclaves #2, #3, and #4. The Laboratory does not run kill loads in autoclave #1.

10.3.2 Verify short gravity cycles (used for sterilizing dry goods), and short liquid runs (used to sterilize liquid media) in autoclaves #1 and #4. Dry goods and liquid media are autoclaved predominately in autoclaves #1 and #4.

10.4 Quarterly Performance Verification and Use of Biological Ampules.

10.4.1 Verify short gravity cycles (used for sterilizing dry goods), and short liquid runs (used to sterilize liquid media) in autoclaves #2 and #3. Autoclaves #2 and #3 are rarely used to sterilize dry goods and liquid media.

10.5 Performance Verification Procedures

10.5.1 Autoclave Printout. For each run, record the minimum and maximum temperatures achieved during the "sterilize" portion of the cycle as indicated on the autoclave printer readout on the appropriate form (see 16.0).

10.5.2 Maximum Registering Thermometer. Place each thermometer upright in a beaker and place in the center of an autoclave pan.

10.5.2.1 For autoclaves #1 and #2, place one pan containing a thermometer in the center of the top shelf and one in the center of the bottom shelf.

10.5.2.2 For autoclave #3 and #4, place one pan containing a thermometer in the center of the bottom shelf.

10.5.2.3 Following the autoclave cycle, record the results on the appropriate forms (see 16.0). The thermometer should be allowed to cool to ambient temperature before it is read (see 5.1).

10.5.2.4 Reset the maximum registering thermometer prior to each use by "shaking" the thermometer as you would a fever thermometer. This will force the mercury through the constriction located above the bulb (see 14.1).

10.5.3 Chemical Indicator Strip.

10.5.3.1 For short liquid runs, place a strip within a rack of

media between tubes.

10.5.3.2 For verifying short gravity cycles and kill load liquid cycles, place strip flat on top (along the lip) of each beaker that contains a maximum registering thermometer (not upright within the beaker).

10.5.3.3 Record the results from the chemical indicator strips on the appropriate form (see 16.0).

10.5.4 Biological Indicator Ampules.

10.5.4.1 Place a biological indicator ampule into a test tube containing an appropriate volume of liquid (10 mL for 20×150 mm test tubes and 20 mL for 25×150 mm test tubes). Place the test tube containing the ampule in a test tube rack containing 39 other similarly filled test tubes (each rack holds 40 test tubes).

10.5.4.2 Place the tube with the biological indicator ampule as close to the center of the rack as possible. Place this rack into an autoclave pan.

10.5.4.3 For autoclaves #1 and #2, place 2 pans, each containing an ampule as described in 10.3.4.1, in each unit, one in the center of the top shelf and one in the center of the bottom shelf.

10.5.4.4 For autoclave #3 and #4, place one pan containing an ampule as described in 10.3.4.1 in the center of the bottom shelf.

10.5.4.5 Run a gravity cycle or a liquid cycle for the minimum time necessary to kill all spores as indicated with the QC documentation that accompanies each lot of biological ampules (typically from 15 to 20 minutes).

10.5.4.6 Immediately upon completion of the cycle, remove

the pans from the autoclave.

10.5.4.7 Remove the ampule from the test tube and label (location in autoclave). Incubate the ampule as well as one control ampule that has not been autoclaved at 55-60°C for 48 hours and record the results on the appropriate form (see 16.0).

10.5.4.8 To verify kill loads, place a biological indicator ampule in the center of an autoclave bag filled with solid waste. Run a standard kill load (3 hour liquid cycle). After completion of the cycle, recover and label the ampule and incubate for 48 hours at 55-60°C along with a control ampule that has not been autoclaved. Record the results on the appropriate form (see 16.0).

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink. Information on daily autoclave runs will be recorded on the Daily Sterilization Record Information Form (see 16.1). Information on monthly and quarterly QC autoclave runs will be recorded on the Monthly/Quarterly Sterilization Record Information Form (see 16.2). Completed forms are archived in notebooks kept in locked file cabinets in file room D217. Only authorized personnel have access to the locked files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

12.2 The autoclave printouts will be collected periodically and stored in room D217.

13.0 QUALITY CONTROL:

13.1 The OPP Microbiology Laboratory conforms to 40CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Refer to Table 1 for a complete discussion of nonconformance and corrective action scenarios.

14.2 If a processed biological indicator ampule fails (i.e., growth after incubation at 55°C for 48 hours), another ampule should be processed at the same parameters. If the nonconformance persists, service should be scheduled and the autoclave should not be used.

Table 1

Temperature		Chem Strip	
Max	Unit		
P	P	F	Check the location of the Chem Strip†. Check that the chem strip for the next cycle passes. If the nonconformance pattern persists, run a cycle and process a biological indicator. Incubate and record the results of the biological indicator (see 14.2). *Check sterility of media and use if it passes. Repeat the cycle for glassware/dry goods.*
F	P	P	If a maximum registering thermometer deviates from the sterilize set temperature by greater than 3°C, the cycle is considered to be in nonconformance. Repeat the cycle to verify the nonconformance. If the nonconformance pattern persists, verify the nonconformance again using a different maximum registering thermometer. If the nonconformance persists, run a cycle and process a biological indicator (see 14.2). Call for service. *Check sterility of media and use if it passes. Repeat the cycle for glassware/dry goods.*
F	P	F	Check the location of the Chem Strip†. Repeat the cycle to verify the nonconformance. If the nonconformance pattern persists, verify the nonconformance again using a different maximum registering thermometer. If the nonconformance persists, run a cycle and process a biological indicator (see 14.2). *Check sterility of media and use if it passes. Repeat the cycle for glassware/dry goods*.
P	F	F	The autoclave alarms have been set to trip when the sterilize temperature falls below the sterilize set point (121°C) by more than 1°C. The under temperature alarms will occur during the sterilize phase of the cycle whenever the chamber drain temperature falls below 120°C. If the alarm occurs, an alarm will sound and an under temperature message will be printed. If the chamber drain temperature rises above the alarm set point (120°C), the alarm will silence and another message will be printed. The autoclaves are set to resume the sterilize phase once the temperature falls within acceptable limits. Consider the unit temperature to have passed and see the appropriate nonconformance if the Maximum registering thermometer or the Chem strip fail. If the total sterilize phase lasts longer than the set time by more than 15 minutes, the Too Long in Sterilize alarm will sound and the cycle will be aborted. A message will be printed and the LED readout will flash "Component Failure." Call for service. *If the autoclave goes into alarm but the cycle resumes and is completed successfully, the media should be checked for sterility and performance and can be used if it passes. Repeat the cycle for glassware/dry goods. If the "Too Long in Sterilize" alarm is tripped, media should be discarded and glassware/dry goods should be autoclaved again.*
P	F	P	
F	F	F	
		P	

* Italics indicates corrective action to be taken in regards to media being processed at the time the original nonconformance occurred.
† The Chem Strip should be placed in a location that is fully exposed to the steam. It should not be placed within the beaker that holds the Max. thermometer. If the load includes racks and tubes of media, the Chem Strip should be placed within the rack between tubes.

15.0 REFERENCES:

15.1 Bordner, R.H., Winter, J.A., and Scarpino, P.V., eds. 1978. Microbiological Methods for Monitoring the Environment, Water and Wastes. EPA 600/8-78-017, Environmental Monitoring & Support Lab., U.S. Environmental Protection Agency, Cincinnati, Ohio.

15.2 Eaton, A.D., Clesceri, L.S., Greenberg, A.E. eds. 1995. Standard Methods for the Examination of Water and Wastewater, 19th Edition. American Public Health Association, American Water Works Association, Water Environment Federation.

15.3 Lee, C.-H., Montville, T.J., and Sinskey, A.J., 1979. Comparison of the efficacy of steam sterilization indicators. Appl. Environ. Microbiol. 37(6):113-117.

16.0 FORMS AND DATA SHEETS:

16.1 Daily Sterilization Record Information Form

16.2 Monthly/Quarterly Sterilization Record Information Form

Daily Sterilization Record Information Log Form

OPP Microbiology Laboratory

Cycle[1]		Unit Temp.[2]		Max. Thermometer[3]		Chemical Indicator Strips			Items Processed	Sterilization No.[5]	Init.
Type	Time	Min.	Max.	Temp.	Serial #	Lot No.	Exp. Date	Result[4]			

1 Record the cycle as “G” = gravity, “L” = liquid under Type and the duration of the cycle in minutes under Time.
2 Record the maximum and minimum temperature achieved during the sterilize phase of the cycle as indicated by the autoclave printout (Unit).
3 Record the corrected value for the maximum registering thermometer (Max.) and the serial number of the thermometer.
4 Record the results of the chemical indicator strips as “P” for pass or “F” for fail.
5 The Sterilization No. indicates the date as well as the unit location and the run number.

Monthly/Quarterly Sterilization Record Information Form

OPP Microbiology Laboratory

STERILIZER ID AND INFORMATION																
Chem. Strip Lot No./Exp. Date								Bio. Amp. Lot No./Exp. Date								
Room and Unit ID	Cycle[1]		Biological Ampule Results[2]			Temperature (°C)						Chem. Strip Results		Sterilization Run No.[5]	Init.
						Max. Registering Thermometer[4]				Unit[3]					
						Top	Bottom	Serial #		Max.	Min.				
	Type	Time	Top	Bottom	C			Top	Bottom			Top	Bottom		
B206 #1	G														
	L														
	Kill[6]														
B204 #2	G														
	L														
	Kill[6]														
B207 #3	G														
	L														
	Kill[6]														
B202 #4	G														
	L														
	Kill[6]														

1 Record the cycle as "G" = gravity, "L" = liquid under Type and the duration of the cycle in minutes under Time.
2 Record the results of the ampules after incubation as growth (+) or no growth (0) for the autoclaved ampule (Growth) and unautoclaved control (C).
3 Record the maximum and minimum temperature achieved during the sterilization phase as indicated by the autoclave printer (Unit)
4 Record the corrected value for the maximum registering thermometer and the serial number of the thermometer.
5 The Sterilization No. indicates the date as well as the unit location and the run number.
6 Record the results for the analysis of kill loads (see 10.3.4.8). If not performed, put an ND, "not done."